Structure–Activity Study of Antihypertensive 1,4-Dihydropyridine Derivatives Having Nitrooxyalkyl Moieties at the 3 and 5 Positions[1)]

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Research Center, Taisho Pharmaceutical Co., Ltd.,[a] Yoshino-cho, Ohmiya, Saitama 330, Japan and School of Pharmaceutical Sciences, Kitasato University,[b] Shirokane 5-chome, Minato-ku, Tokyo 108, Japan.
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1,4-Dihydropyridine derivatives having two nitrooxyalkyl moieties as esters at the 3 and 5 positions possess antihypertensive activity. To understand how substituents affect the biological activity, the quantitative structure–activity relationship (QSAR) of 27 compounds was analyzed using the Fuzzy adaptive least-squares (FALS 91) method. The QSAR models suggested that the hydrophobicity and electronic effect at the 4 position of the 1,4-dihydropyridine along with the special structures of the nitrooxyalkylester components are important for antihypertensive activity.

Keywords 1,4-dihydropyridine; antihypertensive activity; structure–activity relationship; FALS method

4-Aryl-1,4-dihydropyridine derivatives such as nifedipine and nicardipine have a high affinity for the voltage-dependent calcium channel, which results in their potent vasodilator effects.[2)] Organic nitrate compounds including nitroglycerin, isosorbide dinitrate, and nicolandil increase the level of cyclic guanosine 5′-monophosphate (cyclic GMP) produced in various vascular smooth muscle tissues and promote relaxation.[3)] Simultaneous use of calcium antagonist and nitrate compounds clinically enhances the therapeutic antihypertensive effect with few side-effects. The combination of nitro-like and calcium-blocking actions in a single molecule was therefore expected to yield a new class of potent vasodilators, other than the known 1,4-dihydropyridines.

In the previous papers,[4,5)] we reported the synthesis and antihypertensive activity of 4-substituted 1,4-dihydropyridine-3,5-dicarboxylic acid derivatives having nitrooxy moieties. Antihypertensive activities of the 1,4-dihydropyridine derivatives having two nitrooxyalkyl moieties as esters at the 3 and 5 positions were shown to be more potent than those of the compounds having one nitrooxyalkyl moiety in the molecule.

Among the derivatives having two nitrooxyalkyl moieties, introduction of pyridine and thiophene groups at the 4 position of 1,4-dihydropyridine decreased the activities. The replacement of these groups by a nitrophenyl group increased the activities. These considerations prompted us to synthesize various derivatives and attempt a quantitative structure–activity analysis (QSAR). The QSAR was done by using the Fuzzy adaptive least-squares (FALS) method[6,7)] for 27 compounds including the newly synthesized compounds.

Chemical Synthesis and Assay To examine further the effect of various substituents on the benzene ring, four derivatives having 2- and 3-trifluoromethyl and 3,4-dichloro groups on the phenyl ring were synthesized and evaluated for ability to increase blood flow. The compounds listed in Table I were synthesized by the method reported in the previous paper[4)] *via* either the Hantzsch reaction (method A) or a modified reaction (method B), or through the esterification reaction of 1,4-dihydropyridine mono- or dicarboxylic acid with alcohols or alkyl bromides (methods C and D). The method of synthesis are summarized in Chart 1.

Antihypertensive activity of newly synthesized compounds was evaluated by examining the effects on femoral arterial blood flow (FBF) and vertebral arterial blood flow (VBF) in anesthetized dogs. The biological data are shown in Table I.

The newly synthesized compounds showed high antihypertensive activities compared with nifedipine. The activities of the compounds having trifluoromethylphenyl and chlorophenyl groups were greater than those of the compounds having pyridine and thiophene groups at the 4 position.

QSAR of 1,4-Dihydropyridine Derivatives (27 Compounds) The compounds analyzed in the QSAR study are listed in Table I, along with the structural descriptors and activity ratings. In the parametrization of structural features for the FALS study, we investigated physicochemical parameters generally used in QSAR studies and indicator variables. In the FALS analysis using these descriptors, good discriminant functions were derived as Eqs. 1—3 for FBF activity and Eqs. 4 and 5 for VBF activity (Table II).

In Eqs. 1—5, π_R is the hydrophobic parameter estimated using the fragment method reported by Leo *et al.*[8)] The values of Q (multiplied by 100) are the electronic charge at the 4 position of the dihydropyridine ring to which the substituent (R) is bound. They were calculated using a complete neglect of differential overlap (CNDO) molecular orbital calculation program. The indicator variables D_1 and D_2 were assigned a value of 1 corresponding to the presence of special structures of the nitrooxyalkyl moieties, A and B (A B in chain length): D_1 is for A=$-CH_2-CH_2-$ or $-CH(CH_3)-CH_2-$ and B=$-(CH_2)_3-$, and D_2 is for A=B=$-(CH_2)_3-$. The figure in parentheses under the coefficient is the contribution index (=|coefficient|×S.D. of the descriptor), which is a measure of the contribution of the descriptor to the discriminant score (Z). The squared cross-correlation matrix of the descriptors used in Eqs. 1—5 is shown in Table III. There seems to be no problem due to cross-correlation between descriptors in these equations.

In these equations, greater values of the contribution index for π_R may indicate that the hydrophobicity of substituent R is important. Further, an electron-withdrawing substituent R is favorable to the activity. Among

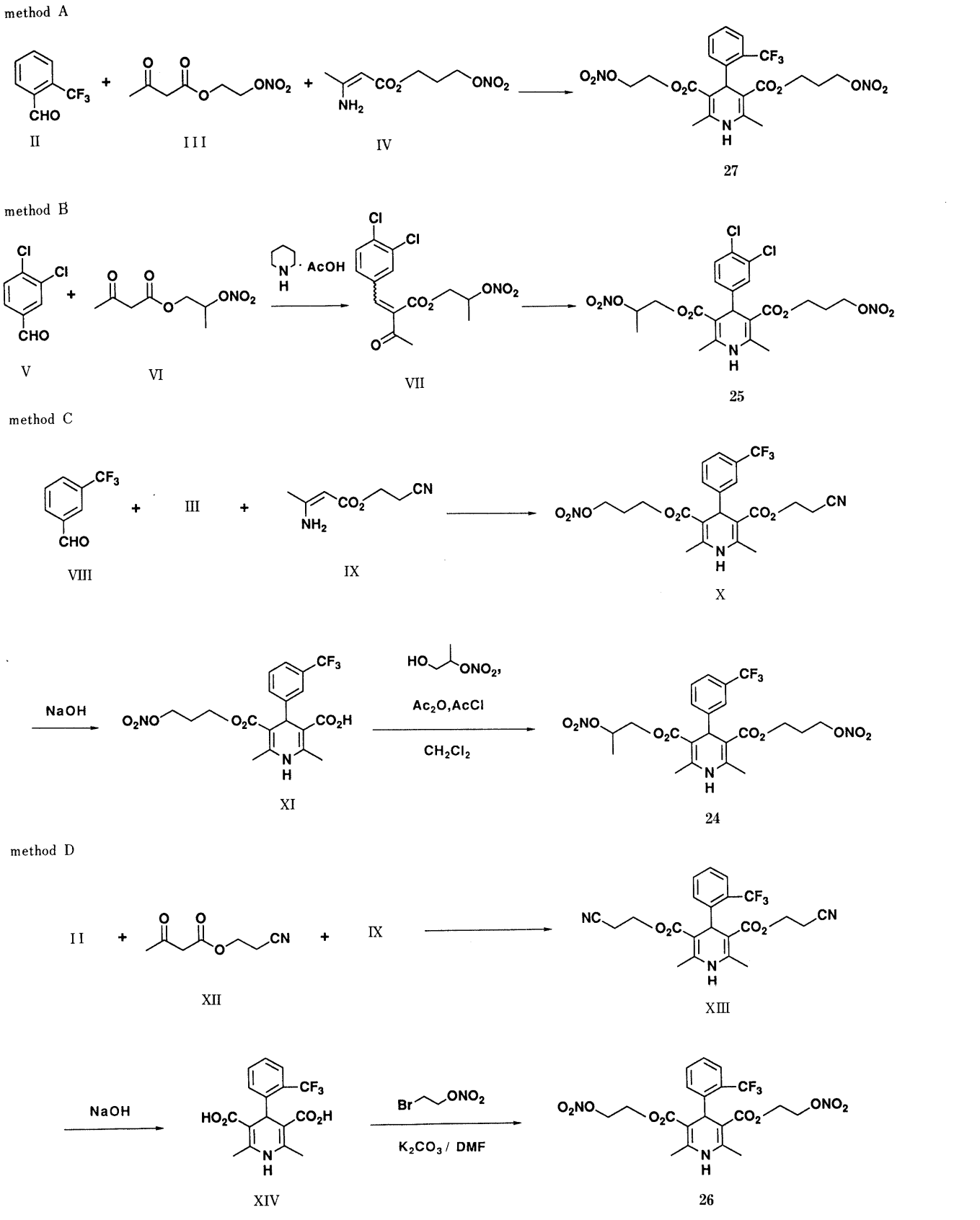
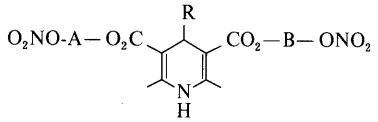


Chart 1

the indicator variables, the positive coefficient for D_1 and the negative coefficient for D_2 may indicate positive and negative preference of the corresponding structures of the nitrooxyalkyl moieties for activity, respectively. This may correspond to the finding by Satoh *et al.*[9] that asymmetrical esters at the 3 and 5 positions were somewhat more effective than symmetrical esters in 2-hydroxymethyl- and 2-cyano-1,4-dihydropyridines. The reason for this is unknown,

TABLE I. Structure–Activity, and Descriptors for 1,4-Dihydropyridine Derivatives Having Two Nitrooxyalkyl Moieties



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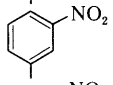
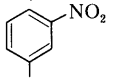
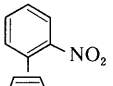
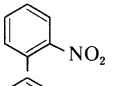
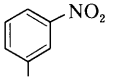
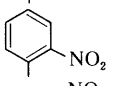
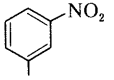
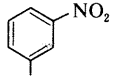
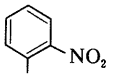
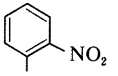
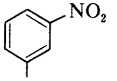
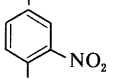
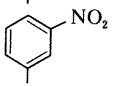
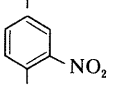
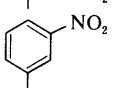
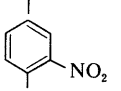
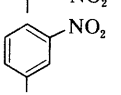
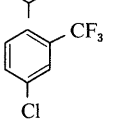
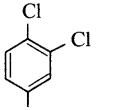
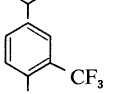
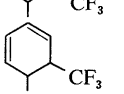
No.	A	B	R	FBF activity				VBF activity				π_x	Q	D_1	D_2
				Obs.[a]	Rating.[b]	Recog.[c]	Pred.[d]	Obs.[a]	Rating.[e]	Recog.[f]	Pred.[d]				
1	$-CH_2CH_2-$	$-CH_2CH_2CH_2-$		0.2	1	1	2	0.3	1	1	1	0.66	3.29	1	0
2	$-CH_2CH_2CH_2-$	$-CH_2CH_2CH_2-$		0.2	1	1	1	0.6	2	1	1	0.66	8.11	0	1
3	$-CH_2CH_2CH_2-$	$-CH_2CH_2CH_2-$		0.3	1	1	1	0.7	2	2	2	1.87	0.50	0	1
4	$-CHCH_2-$ (g) CH_3	$-CH_2CH_2CH_2-$		0.4	1	1	1	0.4	1	2	2	1.78	−4.32	1	0
5	$-CH_2CH_2-$	$-CH_2CH_2CH_2-$		0.4	1	1	1	0.4	1	1	1	0.66	−2.46	1	0
6	$-CH_2CH_2CH_2-$	$-CH_2CH_2CH_2-$		0.4	1	1	1	1.1	2	2	2	1.87	1.77	0	1
7	$-CH_2CH_2-$	$-CH_2CH_2CH_2-$		0.5	1	1	1	0.2	1	2	2	1.78	−4.32	1	0
8	$-CH_2CH_2CH_2CH_2-$	$-CH_2CH_2CH_2CH_2-$		0.7	2	2	2	0.4	1	2	2	1.87	0.50	0	0
9	$-CH_2CH_2-$	$-CH_2CH_2-$		0.8	2	2	2	1.4	3	2	2	1.87	1.77	0	0
10	$-CH_2CH_2-$	$-CH_2CH_2CH_2-$		0.9	2	2	2	0.2	1	1	1	0.66	8.11	1	0
11	$-CHCH_2-$ CH_3	$-CH_2CH_2-$		1.0	2	2	2	1.0	2	2	2	1.87	0.50	0	0
12	$-CH_2CH_2-$	$-CH_2CH_2CH_2CH_2-$		1.0	2	2	2	1.0	2	2	2	1.87	1.77	0	0
13	$-CH_2CH_2-$	$-CH_2CH_2CH_2CH_2-$		1.0	2	2	2	1.0	2	2	2	1.87	0.50	0	0

14	–CH_2–$\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}$–[h]	–$CH_2CH_2CH_2$–		1.1	2	2	2	0.8	2	2	2	1.87	0.50	0	0
15	–$CH_2CH_2CH_2CH_2$–	–$CH_2CH_2CH_2CH_2$–		1.2	2	2	2	0.6	2	2	2	1.87	1.77	0	0
16	–CH_2–$\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}$–	–$CH_2CH_2CH_2$–		1.4	2	2	2	0.6	2	2	2	1.87	1.77	0	0
17	–CH_2CH_2–	–CH_2CH_2–		1.4	2	2	2	1.0	2	2	2	1.87	0.50	0	0
18	–$CH_2CH_2CH_2$–	–$CH_2CH_2CH_2CH_2$–		1.5	3	2	2	1.5	3	2	2	1.87	1.77	0	0
19	–$\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}$–$CH_2$–	–$CH_2CH_2CH_2$–		1.5	3	3	2	1.3	3	3	3	1.87	0.50	1	0
20	–CH_2CH_2–	–$CH_2CH_2CH_2$–		1.6	3	3	3	1.3	3	3	3	1.87	1.77	1	0
21	–CH_2CH_2–	–$CH_2CH_2CH_2$–		1.6	3	3	2	1.3	3	3	3	1.87	0.50	1	0
22	–$\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}$–$CH_2$–	–$CH_2CH_2CH_2$–		1.7	3	3	3	1.5	3	3	3	1.87	1.77	1	0
23	–$CH_2CH_2CH_2$–	–$CH_2CH_2CH_2CH_2$–		2.0	3	2	2	1.0	2	2	2	1.87	0.50	0	0
24	–$\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}$–$CH_2$–	–$CH_2CH_2CH_2$–		1.5	3	3	3	1.3	3	3	3	2.19	0.30	1	0
25	–$\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}$–$CH_2$–	–$CH_2CH_2CH_2$–		1.5	3	3	3	1.4	3	3	3	3.54	1.66	1	0
26	–CH_2CH_2–	–CH_2CH_2–		1.5	3	3	3	1.5	3	3	3	2.19	3.03	0	0
27	–CH_2CH_2–	–$CH_2CH_2CH_2$–		1.6	3	3	3	1.4	3	3	3	2.19	3.03	1	0
	Nifedipine			1.0											

a) Relative potency (*RP*) to nifedipine. *b*) Rating 1: $RP \leqq 0.5$; rating 2: $0.5 < RP < 1.5$; rating 3: $RP \geqq 1.5$. *c*) From Eq. 3. *d*) Using the leave-one-out technique. *e*) Rating 1: $RP \leqq 0.4$; rating 2: $0.4 < RP < 1.4$; rating 3: $RP \geqq 1.4$. *f*) From Eq. 4. *g*) –(COO)–$\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}$–$CH_2$–($ONO_2$). *h*) –(COO)–$CH_2$–$\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}$–($ONO_2$).

TABLE II. FALS Discriminant Functions and Their Recognition and Prediction Results

Eq. No.	$n=27$[a]	Recognition			Prediction[b]		
		n_{mis}[c]	R_S[d]	MMG[e]	n_{mis}[c]	R_S[d]	MMG[e]
FBF							
(1)	$Z=2.00\pi_R+0.28Q+1.24D_1-4.53$ (CI=1.14) (0.74) (0.62)	4 (0)[f]	0.873	0.852	7 (0)	0.794	0.740
(2)	$Z=1.18\pi_R+0.10D_1-1.45D_2-1.76$ (CI=0.67) (0.05) (0.46)	5 (0)	0.847	0.782	5 (0)	0.847	0.803
(3)	$Z=1.99\pi_R+0.27Q+0.44D_1-1.59D_2-3.74$ (CI=1.13) (0.71) (0.22) (0.50)	2 (0)	0.937	0.917	5 (0)	0.843	0.793
VBF							
(4)	$Z=1.25\pi_R+0.29D_1-2.13$ (CI=0.71) (0.14)	6 (0)	0.804	0.735	6 (0)	0.804	0.727
(5)	$Z=1.97\pi_R+0.27Q+0.46D_1-3.77$ (CI=1.12) (0.72) (0.23)	4 (0)	0.863	0.844	8 (0)	0.701	0.695

a) Number of points used for calculations. *b*) Using the leave-one-out technique. *c*) Number of misclassified compounds. *d*) Spearman rank correlation coefficient with a correction of many ties; the values are all significant at $p<0.01$. *e*) Mean membership grade. *f*) Number of compounds misclassified by two grades. CI: contribution index.

TABLE III. Cross-Correlation Matrix of Descriptors for Eq. 1—5

	π_R	Q	D_1	D_2
π_R	1.00			
Q	−0.29	1.00		
D_1	−0.06	−0.16	1.00	
D_2	−0.20	0.29	−0.32	1.00

however. The descriptors have the same positive or negative sign in the equations for FBF activity as in those for VBF activity. This is reasonable in view of the significant correlation between FBF and VBF activities ($r=0.69$).

The validity and predictive power of the descriptor sets were investigated by the leave-one-out technique, which reconstructed the QSAR model by removing each compound in turn and predicting the results for the removed compound. The QSAR models gave good predictive results. Among the derivatives having two nitrooxyalkyl moieties, 2-nitrooxypropyl 3-nitrooxypropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylate (CD-349) showed pronounced biological activities. This compound is being further studied.

Experimental

Melting points were determined on Yanagimoto micro melting points apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO DS-301 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-200 (200 MHz) spectrometer using tetramethylsilane as an internal standard. Chemical shifts are given in ppm. The following abbreviation are used: s=singlet, t=triplet, q=quartet, m=multiplet, br=broad. Mass spectra (MS) were measured on a Shimadzu LKB 9000 spectrometer. Column chromatography was performed on 70—230 mesh silica gel from Merck.

General Procedure for the Synthesis of Dihydropyridine (I). 2-Nitrooxyethyl 3-Nitrooxypropyl 1,4-Dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylate (27) Method A: A solution of 2-trifluoromethylbenzaldehyde (II) (1.74 g, 10 mmol), 2-nitrooxyethyl acetoacetate (III) (1.91 g, 10 mmol), and 3-nitrooxypropyl 3-aminocrotonate (IV) (2.04 g, 10 mmol) in 2-propanol (30 ml) was refluxed for 3 h with stirring. The solvent was removed and the resulting residue was purified by chromatography on silica gel with *n*-hexane–AcOEt (1 : 1, v/v) to give **27** (2.21 g, 41.4%) as a pale yellow oil. MS *m/z*: 534 (M^+). IR (neat) cm^{-1}: 3350 (NH). 1701 (C=O). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.21 (2H, m), 2.33 (3H, s), 2.34 (3H, s), 4.12 (2H, m), 4.25 (2H, m), 4.30 (2H, t, $J=6$ Hz), 4.60 (2H, t, $J=5$ Hz), 5.62 (1H, s), 5.73 (1H, br s), 7.10—7.59 (4H, m). *Anal.* Calcd for $C_{21}H_{22}F_3N_3O_{10}$: C, 47.19; H, 4.15; N, 7.86. Found: C, 47.57; H, 4.26; N, 7.72.

2-Nitrooxypropyl 3-Nitrooxypropyl 4-(3,4-Dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate (25) Method B: A solution of 3,4-dichlorobenzaldehyde (3.50 g, 20 mmol), 2-nitrooxypropyl acetoacetate (4.10 g, 20 mmol), AcOH (0.24 g, 4 mmol), and piperidine (0.34 g, 4 mmol) in benzene (200 ml) was refluxed for 2 h with continuous removal of water by a Dean–Stark apparatus. The solvent was removed and the residue was purified by column chromatography on silica gel with *n*-hexane–AcOEt (1 : 1, v/v) as an eluent to give 2-nitrooxypropyl 2-(3,4-dichlorobenzylidine) acetoacetate (VII) (5.60 g, 77.3%) as a pale yellow oil. The ratio of isomers was 2 : 1 from the NMR spectrum. MS *m/z*: 363 $(M+H)^+$. IR (neat) cm^{-1}: 1736, 1703 (C=O). A solution of VII (3.62 g, 10 mmol) and 3-nitrooxypropyl 3-aminocrotonate (IV) (2.04 g, 10 mmol) in 2-propanol (30 ml) was refluxed for 3 h with stirring. After removal of the solvent, the resulting residue was purified by column chromatography on silica gel with *n*-hexane–AcOEt (1 : 1, v/v) as an eluent to yield the 1 : 1 diastereomer **25** (3.76 g, 68.6%) as a pale yellow oil. MS *m/z*: 549 $(M+H)^+$. IR (neat) cm^{-1}: 3342 (NH). 1699 (CO). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.30, 1.35 (3H, each d, $J=7$ Hz), 2.06 (2H, m), 2.35, 2.37 (3H, each s), 4.14 (2H, m), 4.20 (2H, m), 4.43 (2H, m), 4.89 (1H, s), 5.30 (1H, m), 5.79 (1H, br s), 7.05—7.33 (3H, m). *Anal.* Calcd for $C_{21}H_{23}Cl_2N_3O_{10}$: C, 46.00; H, 5.04; N, 7.66. Found: C, 45.71; H, 4.99; N, 7.55.

2-Nitrooxypropyl 3-Nitrooxypropyl 1,4-Dihydro-2,6-dimethyl-4-(3-trifluoromethylphenyl)-3,5-pyridinedicarboxylate (24) Method C: Cyanoethyl 3-nitrooxypropyl 1,4-dihydro-2,6-dimethyl-4-(3-trifluoromethylphenyl)-3,5-pyridinedicarboxylate (X) was similarly prepared from 3-trifluoromethylbenzaldehyde, 3-nitrooxypropyl acetoacetate and cyanoethyl 3-aminocrotonate by method A. Yield 77.2%, mp 73—75 °C (from CH_2Cl_2–hexane). MS *m/z*: 497 (M^+). IR (KBr) cm^{-1}: 3344 (NH). 1698 (C=O). ^{1}H-NMR (200 MHz, $CHCl_3$) δ: 2.02 (2H, m), 2.36 (3H, s), 2.38 (2H, s), 2.66 (2H, t, $J=5$ Hz), 4.13 (2H, m), 4.27 (2H, t, $J=5$ Hz), 4.36 (2H, t, $J=6$ Hz), 5.04 (1H, s), 6.01 (1H, br s), 7.29—7.58 (4H, m). *Anal.* Calcd for $C_{22}H_{22}F_3N_3O_7$: C, 53.12; H, 4.56; N, 8.45. Found: C, 53.40; H, 4.41; N, 8.42.

Subsequently, a suspension of X (49.74 g, 0.1 mol) in acetone (400 ml) and 1 N sodium hydroxide (200 ml) was stirred at room temperature for 2 h. The reaction mixture was diluted with water (200 ml), and extracted with CH_2Cl_2. The aqueous layer was acidified with phosphoric acid (24.50 g, 0.25 mol) under ice cooling. The precipitated product was filtered off, washed with water, and then dried *in vacuo* to give 3-(3-nitrooxypropoxy carbonyl)-4-(3-trifluoromethylpheyl)-1,4-dihydropyridine-5-carboxylic acid (XI) as yellow crystals (38.9 g, 87.5%), mp 144—146 °C (MeOH–Et_2O). MS *m/z*: 444 (M^+). IR (KBr) cm^{-1}: 3324 (NH). 1683 (C=O). ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.94 (2H, m), 2.27 (3H, s), 2.31 (3H, s), 2.31 (3H, s), 4.04 (2H, m), 4.38 (2H, t, $J=6$ Hz), 4.94 (1H, s), 7.38—7.56 (4H, m), 9.92 (1H, br s), 11.88 (1H, br s). *Anal.* Calcd for $C_{19}H_{19}F_3N_2O_7$: C, 51.55; H, 4.31; N, 6.30. Found: C, 51.62; H, 4.29; N, 6.14.

Acetic anhydride (3.06 g, 30 mmol) was added to a suspension of XI (4.44 g, 10 mmol) in CH_2Cl_2 (30 ml) was added at room temperature. A solution of 2-nitrooxypropanol (1.45 g, 12 mmol) in CH_2Cl_2 containing a catalytic amount of acetyl chloride was added to the reaction mixture at

the same temperature and the whole was stirred for 6 h, then diluted with water. The CH_2Cl_2 layer was separated and washed with 1 N aqueous NaOH, then with water, dried (Na_2SO_4), and concentrated. The resulting residue was chromatographed on silica gel (200 g) with *n*-hexane–AcOEt (1 : 1, v/v) to give the 1 : 1 diastereomer 24 (3.86 g, 70.6%) as a yellow oil. MS *m/z*: 547 (M^+). IR (neat) cm^{-1}: 3343 (NH). 1701 (C=O). ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.27, 1.32 (3H each, d, J=6 Hz), 2.05 (2H, m), 2.35 (3H, s), 2.38 (3H, s), 4.16 (2H, m), 4.21 (2H, m), 4.36 (2H, m), 4.99 (1H, s), 5.30 (1H, m), 5.82 (1H, br s), 7.27—7.65 (4H, m). *Anal.* Calcd for $C_{22}H_{24}F_3N_3O_{10}$: C, 48.26; H, 4.42; N, 7.68. Found: C, 48.55; H, 4.11; N, 4.41.

Bis(2-nitrooxyethyl) 1,4-Dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylate (26) Method D: Bis(2-cyanoethyl)-1,4-dihydro-2,6-dimethyl-4-(2-trifluoromethylphenyl)-3,5-pyridinedicarboxylate (XIII) was prepared from 2-trifluoromethylbenzaldehyde (II), 2-cyanoethyl acetoacetate (XII), and cyanoethyl 3-aminocrotonate (IX) by method A. Yield 50.2%, mp 108—109 °C (from CH_2Cl_2–Et_2O). MS *m/z*: 447 (M^+). IR (KBr) cm^{-1}: 3350 (NH), 1698 (C=O). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.35 (6H, s), 2.64 (4H, t, J=7 Hz), 4.23 (4H, m), 5.56 (1H, s), 6.17 (1H, br s), 7.21—7.56 (4H, m). *Anal.* Calcd for $C_{22}H_{20}F_3N_3O_4$: C, 59.06; H, 4.51; N, 9.40. Found: C, 58.93; H, 4.47; N, 9.29.

By following the same procedure as described for the synthesis of XI, removal of the cyanoethyl protecting group in XIII with sodium hydroxide in aqueous acetone gave 2,6-dimethyl-4-(2-trifluoromethylphenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid (XIV). Yield 80.1%, mp 135—137 °C (from MeOH–Et_2O). MS *m/z*: 341 (M^+). IR (KBr) cm^{-1}: 3334 (NH), 1706 and 1675 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 2.22 (6H, s), 5.38 (1H, s), 7.37—7.56 (4H, m), 8.59 (1H, s), 11.56 (2H, br s). *Anal.* Calcd for $C_{16}H_{14}F_3NO_4$: C, 56.31; H, 4.14; N, 4.10. Found: C, 56.20; H, 4.11; N, 3.97.

A solution of XIV (3.41 g, 10 mmol), nitrooxyethyl bromide (3.34 g, 20 mmol) and potassium carbonate (4.15 g, 30 mmol) in dimethyl formamide (DMF) (30 ml) was stirred for 10 h at room temperature. The mixture was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water, and brine, dried ($MgSO_4$), and concentrated. The resulting residue was purified by chromatography on silica gel (200 g) with *n*-hexane–AcOEt (1 : 1, v/v) to give **26** (2.49 g, 48.0%) as a yellow oil. MS *m/z*: 519 (M^+). IR (neat) cm^{-1}: 3420 (NH), 1708 (C=O). ^{1}H-NMR (200 MHz, $CDCl_3$): 2.34 (6H, s), 4.30 (4H, m), 4.60 (4H, t, J=5 Hz), 5.54 (1H, s), 5.78 (1H, br s), 7.17—7.58 (4H, m).

QSAR Analysis Method FALS 91,[6,7] a nonparametric pattern classifier, was developed to correlate structure with activity ratings of compounds. The QSAR model is formulated as a FALS discriminant function that classifies compounds to activity ratings. The discriminant function is generated by a feedback adaptation procedure in a linear formula, Eq. 6:

$$Z = w_0 + w_1x_1 + w_2x_2 + \cdots + w_px_p \quad (6)$$

where Z is the discriminant score, x_k (k=1, 2, ..., p) is the kth descriptor for structure, and w_k is the weight coefficient.

A membership function, $M(Z)$, was assumed to give the grade of membership of classes for compounds. The value of $M(Z)$ (membership grade [MG]) ranged from 0 to 1 and was taken to be 0.5 at the class boundaries. The function for class j can be written as Eq. 7.

$$M(Z) = \begin{cases} 1/[1+\{(Z-b_{j-1})/Fl-1\}^4], & \text{for } Z \leqq b_{j-1}+Fl \\ 1, & \text{for } b_{j-1}+Fl < Z \leq b_j-Fl \\ 1/[1+\{(b_j-Z)/Fl-1\}^4], & \text{for } b_j-Fl < Z \end{cases} \quad (7)$$

where Fl is the parameter for fuzziness of the boundary between classes, generally taken to be 1. The starting score for the members of class j, a_j, is assumed according to Eq. 8, in which ng = size of group g and n_j, and the class boundary, b_j, is taken as the midpoint between any two adjacent starting scores.

$$a_j = 4\left(\sum_{g=1}^{j-1} ng + n_j/2\right)\Big/ n - 2$$

The weight coefficients (discriminant coefficients) in Eq. 6 were determined so as to maximize the sum of membership grades over all samples through two steps of iterative least-squares adaptation. The procedure is described in detail elsewhere.[3]

The results of FALS were validated by leave-one-out prediction. The discriminant function having a scientifically reasonable subset of descriptors and giving the best leave-one-out prediction was finally selected.

Biological Method Male and female mongrel dogs weighing 8—15 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v. supplemented with additional doses as necessary). Female arterial blood pressure was measured with a pressure transducer (Nihon Kohden MPV-0.5, Tokyo, Japan) connected to a rigid polyethylene tube introduced into a femoral artery. Heart rates were measured by a heart rate counter (Nihon Kohden T-600G) driven by the blood pressure pulses. The femoral and vertebral arterial blood flow was measured with an electromagnetic flow-meter probe interposed in an extracorporeal path. The probe was connected to an electromagnetic flow meter (Nihon Kohden MFV-1200). The extracorporeal path was constructed in the femoral and vertebral arteries. The heparinized blood was perfused from the proximal sites of the femoral and vertebral arteries to the distal sites of the arteries through the extracorporeal path. Each test compound dissolved in dimethyl sulfoxide was administered intraarterially *via* the extracorporeal path, and the blood flow was measured by means of the electromagnetic blood flow meter (MF-27, MFV-120, Nihon Kohden, Japan).

References and Notes

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